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STUDIES ON THE COCCACEAE

V. SEROLOGICAL STUDIES OF THE MICROCOCCI

G. J. HUCKER

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STUDIES ON THE COCCACEAE

V. SEROLOGICAL STUDIES OF THE MICROCOCCI

G. J. HUCKER

SUMMARY

A study of a number of representative cultures of the genus Micrococcus indicates that from the standpoint of agglutination, agglutination absorption, and complement fixation, the various species of the micrococci are serologically distinct.

It was found impossible definitely to arrange the species into serological groups. The results of the tests were, however, useful in conjunction with various cultural reactions in arranging a general classification of the genus.

INTRODUCTION

During the last decade, serology has played an important part in the differentiation of various closely related organisms. From the standpoint of classification no survey of any group is complete without some knowledge of the serological reactions of its various members. The importance and application of these results, however, may vary with the particular organisms under study.

It is the purpose of this particular section of the investigation dealing with the species of the genus *Micrococcus*[1] to study the serological relationships of the species as determined by morphological and cultural studies. No attempt will be made to group the micrococci into definite serological groups without reference to other characters, as it is believed that the results of the various serological tests should be dealt with and studied with the same viewpoint as that maintained in considering any other character. Serological tests should be taken into account as the primary basis for separations only when they designate sub-groups containing closely related organisms. In many instances, however, certain strains and varieties can be separated advantageously by serological tests, especially where minor sub-groups are desired in order to make a definite diagnosis.

[1]See Technical Bulletins Nos. 99, 100, 101, and 102 for other sections of these investigations.

PREVIOUS STUDIES

A number of studies of the serological reactions of the micrococci have been presented at various times, either from the standpoint of classification or as a means of determining possible methods of attacking infections which have as their etiological factor certain members of this group.

Landsteiner (1897)[2] was probably the first to produce immune bodies for this group, using rabbits as test animals; while Silvestrini (1898) agglutinated micrococci with human serum from infected patients. Kraus and Löw (1899) in repeating the work of Silvestrini noted that normal human serum may agglutinate various strains of this group in dilutions as high as 1:100. Nicolas and Lesieur (1901) studied certain types with immune sera produced in goats and found that their cultures fell into definite serological groups.

Probably the most extensive study of the serology of this group was that of Neisser and Wechsberg (1901) who showed that pathogenic and saprophytic micrococci might be separated by agglutination and also by the production of hemolysin. Attempts to differentiate micrococci serologically were repeated by Kolle and Otto (1902), Otto (1903), Pröscher (1903, 1903a), Klopstock and Bockenheimer (1904), Fraenkal and Baumann (1905), Walker and Adkinson (1917), Hines (1922), and Jullianelle (1922). From the standpoint of the agglutination test alone all of the above investigators reported distinct differences between the saprophytic forms and those generally associated with pathogenic conditions.

Complement fixation has been used by Ballner and Reibmayr (1907), Altmann and Schultz (1909), and Altmann and Blühdorn (1910) to separate the micrococci into sub-groups and as a means of serologically distinguishing this group from the sarcinae.

Courmont (1894) and Wolf (1896) investigated the development of lytic substances which may be elaborated in the medium, as a basis for a logical separation of the various groups, but their results have been of little aid in the classification of the group.

Mircoli (1894) and Reichenbach (1897) partially confirmed the above work by noting that immune sera of laboratory animals

[2]See Bibliography, page 17.

also evidenced a certain amount of protective action against injections of virulent micrococci.

For a number of years it was believed that the presence or abscence of a hemolyzing substance was an accurate indication of the nature of the micrococci, *viz.*, saprophytic or pathogenic. Kraus (1900), Neisser and Wechsberg (1901), Kraus (1902), Kolle and Otto (1902), Lohr (1905), Axenfeld (1907), Mantegazza (1908), Trincas (1908), Jung (1909), Flügge (1910), Bruck and Hidaka (1911), Jullianelle (1922), and others have endeavored to separate the hemolytic types and correlate this character with parasitism. The general conclusion from a study of these excellent contributions must be that in general no relation was found to exist between the power of hemolyzing red blood cells and the virulence of the organisms.

That some factor, other than habitat, is involved in hemolysis has been shown by Orcutt and Howe (1922) who found that micrococci were only hemolytic in the presence of fats or fat-like substances. They concluded that hemolysis was due to the action of a fatty acid or a soap upon the red cells, the fatty acid probably being formed by the action of the lipase liberated by the organisms.

METHODS

The agglutination, agglutination absorption, and complement fixation tests were adopted as the methods which would probably throw the most light on the serological relationships of the species of this group.

AGGLUTINATION

Preparation of immune serum.—Rabbits were inoculated at intervals of three to five days with saline suspensions of the organisms washed from a 24-hour agar slant. The first two injections were made with suspensions which had been heated to 60° C. for one hour in a water bath, while later unheated cultures were used. All injections were intravenous. The serum was collected by the usual method of bleeding from the lateral ear vein.

Preparation of antigen.—Saline suspensions of the organisms from 12-hour agar slants to which 0.5 per cent phenol had been added were diluted until the suspension compared in density with Standard No. 1 of the MacFarland (1907) nephelometer, and then filtered thru cotton.

Procedure.—Three cc. of antigen were used and sufficient serum added to make the following dilutions: 1:50, 1:100, 1:400, 1:750, 1:1000, 1:1250, 1:1500, 1:2000, 1:2500, 1:3000. The tubes were corked, shaken, and incubated in the 37° C. incubator for 24 hours, shaken, and allowed to stand at room temperature for two days before reading.

Several trials were made in which the incubation was carried on in a 55° C. water bath for two hours followed by 12 hours in the ice chest, but the results were not as clear cut as those secured by the longer incubation procedure.

Positive agglutinations were recorded when the test organisms were clumped at the bottom leaving a clear supernatant fluid.

AGGLUTINATION ABSORPTION

The serum and antigen were prepared as for the agglutination. Four cc. of the antigen were added to 1 cc. of serum, which had been diluted 1:2, and incubated in the 55° C. water bath for two hours followed by 24 hours in the ice chest. The organisms were removed from the suspension by centrifugation and the supernatant diluted serum tested against the homologus and also the absorbing strain. Dilutions were made as in the agglutination test.

COMPLEMENT FIXATION

Preparation of antigen.—The growth from a 12-hour agar slant of a culture which had undergone several rapid transfers was washed off in a saline, taking care to secure only such organisms as were removed easily by gently shaking the tube. The growth was not disturbed with a needle. The suspension was then washed three times in saline and 0.5 per cent tricresol and 1 per cent glycerin added. The suspended organisms were heated in a water bath at 60° to 62° C. for one hour and used in the amounts noted below for antigen in the fixation tests.

The preparation of antigen furnished many difficulties due to the presence of anti-complementary substances. However, the anti-complementary property could be reduced to a minimum if the organisms were washed gently from the slant by shaking the tube with the added saline. In those cases where the growth was scraped off with a needle the antigen showed an anti-complementary action even in low dilutions and in such cases even repeated washings failed to alleviate this

undesirable factor. Little difference could be noted in the action of the antigen following heating at various temperatures ranging from 60° to 70° C.

The antigen was titrated for anti-complementary action and one-third of the smallest amount showing anti-complementary action was used in the fixation tests.

Preparation of immune serum.—The serum was prepared as for the agglutination test and 0.01, 0.005, 0.001, and 0.0005 cc. used in the tests.

Preparation of hemolytic system.—(a) Sheep cells. Five-tenths cc. of a 2 per cent suspension of washed sheep cells was used. (b) Hemolysin. Five units of anti-sheep cell serum were used. (c) Complement. One and one-half units of guinea pig complement were used.

Procedure.—The immune serum and antigen were combined in 2 cc. of saline and inactivated at 56° C. for one hour in a water bath. Following the addition of complement the tubes were incubated in the water bath at 37° C. for one hour. This incubation was repeated after the addition of the hemolytic system.

SELECTION OF ORGANISMS

Typical cultures of the following 15 species described in the previous section of this report (Technical Bulletin No. 102) as probably the most common species of the genus *Micrococcus*, were selected and immune sera prepared for each: *M. albus*, *M. aureus*, *M. aurantiacus*, *M. candidus*, *M. ureae*, *M. epidermidis*, *M. casei*, *M. tetragenus*, *M. luteus*, *M. varians*, *M. flavus*, *M. conglomeratus*, *M. citreus*, *M. roseus*, and *M. cinnebareus*.

Sera were produced of a high titre for each of the above cultures and during the course of the investigation three different rabbits were immunized with each culture.

DATA

AGGLUTINATION

The micrococci have been studied by many investigators from the standpoint of agglutination in an effort to separate this group into sub-groups. As pointed out above, it is doubtful if this test will ever be applicable for dividing the genus into natural sub-groups. It

TABLE 1.—AGGLUTINATION OF THE SPECIES OF MICROCOCCI.

ANTIGEN	IMMUNE SERA AGAINST CULTURE NUMBER														
	385	270	238	111	401	191	88	484	426	80	196	62	167	213	349
	Dilution	*Dilution*	*Dilution*	*Dilution*	*Dilution*	*Dilution*	*Dilution*	*Dilution*	*Dilution*	*Dilution*	*Dilution*	*Dilution*	*Dilution*	*Dilution*	*Dilution*
M. aureus (385)	3000*	400	200	——	0	——	0	——	400	400	0	0	——	–	00
M. aurantiacus (270)	400	2000	400	0	0	——	0	——	——	0	0	50	——	0	0
M. albus (238)	100	0	2000	0	400	0	50	0	0	——	0	0	——	50	50
M. casei (111)	0	0	0	2000	0	0	0	——	——	——	0	0	——	0	0
M. candidus (401)	——	——	——	——	1400	0	——	——	——	——	0	0	——	——	——
M. epidermidis (191)	0	——	400	0	0	2000	50	0	——	——	0	0	——	0	0
M. ureae (88)	0	——	100	——	——	100	3000	——	——	——	0	0	——	0	50
M. tetragenus (484)	——	——	400	——	——	0	——	3000	——	——	——	——	——	——	——
M. luteus (426)	400	——	——	——	——	——	——	——	3000	——	——	——	——	——	——
M. varians (80)	50	——	——	——	——	——	50	——	0	3000	200	0	300	50	0
M. flavus (196)	0	——	0	——	——	——	0	——	——	——	2000	0	0	——	0
M. conglomeratus (63)	0	——	200	——	——	——	50	——	——	——	0	1400	0	50	0
M. citreus (167)	0	0	0	——	——	——	0	——	——	100	50	0	3000	0	0
M. roseus (213)	0	——	0	——	——	——	0	——	——	——	0	0	——	1500	100
M. cinnebareus (349)	0	——	0	——	——	——	0	——	——	——	0	50	——	400	2000

* Figures indicate the highest dilution in which a positive agglutination occurred.
0 Indicates no reaction.
— Indicates test not tried.

evidences in many cases, however, a decided specificity which probably indicates that its chief value lies in separating different species rather than as a character of primary importance in separating the group along broader lines.

It may be noted in Table 1 that the orange cultures, as represented by *M. aureus* and *M. aurantiacus*, are serologically distinct, as far as agglutination is concerned, from the rest of the group. It is interesting to note that in all cases *M. varians*, which appears culturally to be entirely different from *M. aureus*, has shown a certain amount of cross-agglutination when *M. aureus* serum is used. This cross-agglutination was noted in a series of several trials of this particular point. This serological relationship is interesting in light of the commnets regarding the evolutionary development of the micrococci made in a previous section (Technical Bulletin No. 100). If *M. aureus* is of comparatively recent origin from the evolutionary standpoint, it would be expected that a certain amount of group agglutinins would still be produced by this species. There appears to be a quite close relationship between *M. aureus* and *M. aurantiacus* as *M. aurantiacus* cross-agglutinated with *M. aureus* in a dilution of 1:400,while the homologous strain agglutinated in a dilution of 1:2000. Similar results were found when *M. aureus* was used against the *M. aurantiacus* serum.

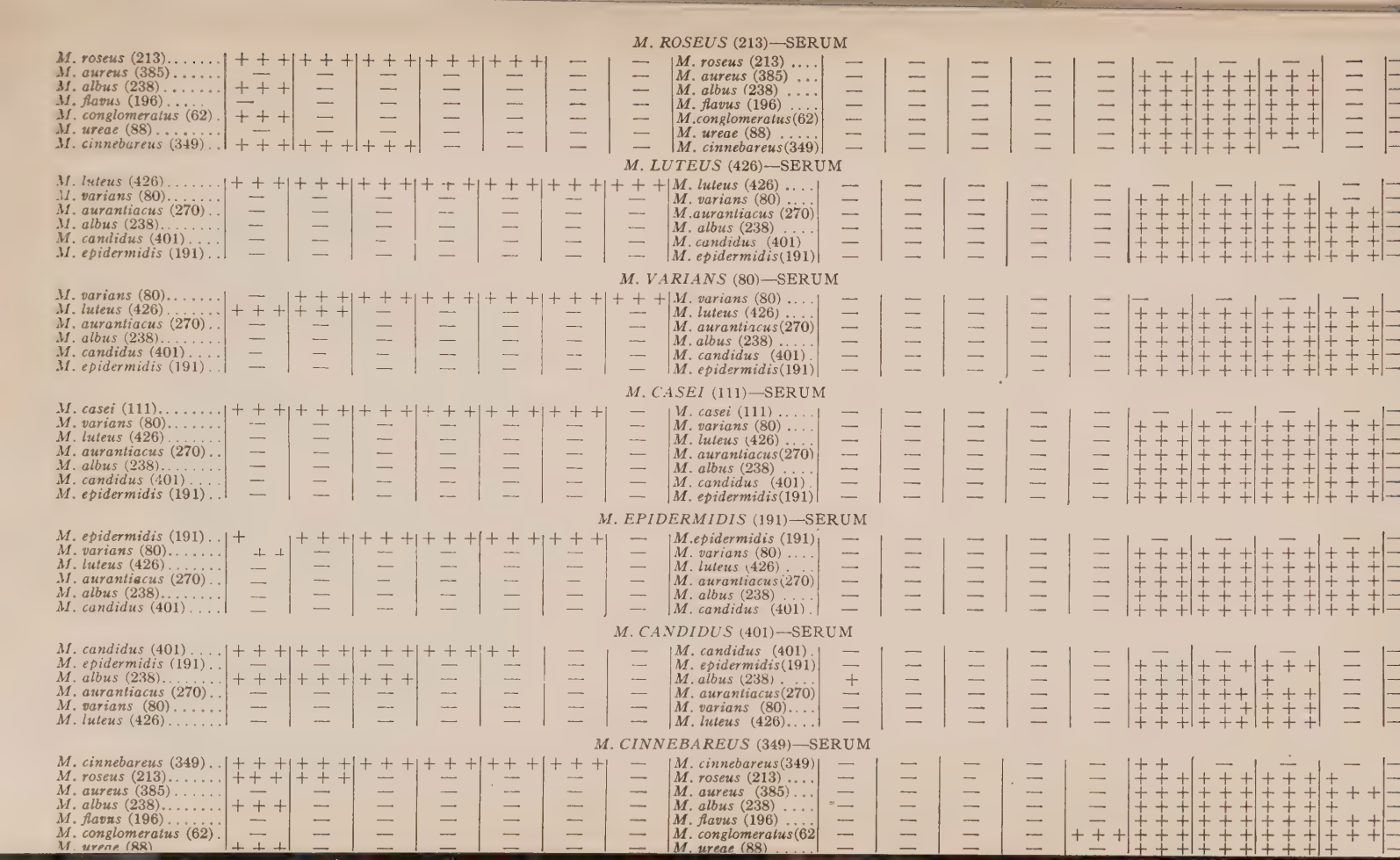
The relationship between *M. aureus* and *M. albus* has always more or less been confused in various laboratories, especially when considered from a serological standpoint. *M. aureus* appears to be serologically distinct from *M. albus* and the conception that *M. albus* is a variety of *M. aureus* which has lost its power to produce pigment is not substantiated by the present results.

The species of white micrococci other than *M. albus* appear to show no cross-agglutination among themselves and very little cross-agglutination with other species, altho in each instance the sera when tested against its own homologous strain had a high titre. Many other strains, altho closely associated culturally with those of white types, gave very little if any cross-agglutination. *M. albus* showed more cross-agglutination with other white strains and with yellow and orange types than did any other species of the white group.

Very little cross-agglutination was noted among the species of the yellow cocci even between cultures which were known to be very

TABLE 2.—STUDIES ON THE AGGLUTINATION ABSORPTION OF VARIOUS SPECIES OF MICROCOCCI.

STRAINS	AGGLUTINATION UNABSORBED SERUM							ABSORBED BY	AGGLUTINATION ABSORBED SERUM										CONTROLS
									ABSORBING STRAIN					SERUM STRAIN					
	50	100	400	750	1500	2000	3000		50	100	400	750	2000	50	400	750	2000		
								M. AUREUS (385)—SERUM											
M. aureus (385)	—	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	*M. aureus* (385)	—	—	—	—	—	—	—	—	—	—	—
M. albus (238)	+ + +	+ +	—	—	—	—	—	*M. albus* (238)	—	—	—	—	—	+	—	—	—	—	—
M. flavus (196)	—	—	—	—	—	—	—	*M. flavus* (196)	—	—	—	—	—	+ + +	+ + +	+ + +	+ +	+	—
M. conglomeratus (62)	—	—	—	—	—	—	—	*M. conglomeratus* (62)	—	—	—	—	—	+ + +	+ + +	+ + +	+ +	—	—
M. ureae (88)	—	—	—	—	—	—	—	*M. ureae* (88)	—	—	—	—	—	+ + +	+ + +	+ + +	+ +	—	—
M. roseus (213)	—	—	—	—	—	—	—	*M. roseus* (213)	—	—	—	—	—	+ + +	+ + +	+ + +	+ +	—	—
M. cinnebareus (349)	—	—	—	—	—	—	—	*M. cinnebareus* (349)	—	—	—	—	—	+ + +	+ + +	+ + +	+ +	—	—
								M. ALBUS (238)—SERUM										
M. albus (238)	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	*M. albus* (238)	+ + +	—	—	—	—	+	—	—	—	—	—
M. aureus (385)	+ + +	+	—	—	—	—	—	*M. aureus* (385)	—	—	—	—	—	+ + +	+ + +	+ + +	+	—	—
M. flavus (196)	—	—	—	—	—	—	—	*M. flavus* (196)	—	—	—	—	—	+ + +	+ + +	+ + +	+ +	+	—
M. conglomeratus (62)	+ + +	+ + +	+	—	—	—	—	*M. conglomeratus* (62)	—	—	—	—	—	+ + +	+ + +	+ + +	+	—	—
M. ureae (88)	+ + +	+ +	—	—	—	—	—	*M. ureae* (88)	—	—	—	—	—	+ + +	+ + +	+ + +	+ +	—	—
M. roseus (213)	—	—	—	—	—	—	—	*M. roseus* (213)	—	—	—	—	—	+ + +	+ + +	+ + +	+ +	—	—
M. cinnebareus (349)	—	—	—	—	—	—	—	*M. cinnebareus* (349)	—	—	—	—	—	+ + +	+ + +	+ + +	+ +	+	—
								M. FLAVUS (196)—SERUM										
M. flavus (196)	—	+ + +	+ + +	+ + +	+ + +	+ + +	+	*M. flavus* (196)	—	—	—	—	—	—	—	—	—	—	—
M. aureus (385)	—	—	—	—	—	—	—	*M. aureus* (385)	—	—	—	—	—	+ + +	+ + +	+ + +	+ +	+	—
M. albus (238)	—	—	—	—	—	—	—	*M. albus* (238)	—	—	—	—	—	+ + +	+ + +	+ + +	+ +	+	—
M. ureae (88)	—	—	—	—	—	—	—	*M. ureae* (88)	—	—	—	—	—	+ + +	+ + +	+ + +	+ +	+	—
M. conglomeratus (62)	—	—	—	—	—	—	—	*M. conglomeratus* (62)	—	—	—	—	—	+ + +	+ + +	+ + +	+ +	+	—
M. roseus (213)	—	—	—	—	—	—	—	*M. roseus* (213)	—	—	—	—	—	+ + +	+ + +	+ + +	+ +	+	—
M. cinnebareus (349)	—	—	—	—	—	—	—	*M. cinnebareus* (349)	—	—	—	—	—	+ + +	+ + +	+ + +	+ +	+	—
								M. CONGLOMERATUS (62)—SERUM										
M. conglomeratus (62)	+ + +	+ + +	+ + +	+ + +	+ + +	—	—	*M. conglomeratus* (62)	—	—	—	—	—	—	—	—	—	—	—
M. aureus (385)	—	—	—	—	—	—	—	*M. aureus* (385)	—	—	—	—	—	+ + +	+ + +	+ + +	—	—	—
M. albus (238)	—	—	—	—	—	—	—	*M. albus* (238)	—	—	—	—	—	+ + +	+ + +	+ + +	—	—	—
M. flavus (196)	—	—	—	—	—	—	—	*M. flavus* (196)	—	—	—	—	—	+ + +	+ + +	+ + +	—	—	—
M. ureae (88)	—	—	—	—	—	—	—	*M. ureae* (88)	—	—	—	—	—	+ + +	+ + +	+ + +	—	—	—
M. roseus (213)	—	—	—	—	—	—	—	*M. roseus* (213)	—	—	—	—	—	+ + +	+ + +	+ + +	—	—	—
M. cinnebareus (349)	+ + +	—	—	—	—	—	—	*M. cinnebareus* (349)	—	—	—	—	—	+ + +	+ + +	+ + +	—	—	—
								M. UREAE (88)—SERUM										
M. ureae (88)	+ + +	+ + +	+ + +	+ + +	+ + +	—	—	*M. ureae* (88)	—	—	—	—	—	—	—	—	—	—	—
M. aureus (385)	—	—	—	—	—	—	—	*M. aureus* (385)	—	—	—	—	—	+ + +	+ + +	+ + +	—	—	—
M. albus (385)	+ + +	—	—	—	—	—	—	*M. albus* (385)	—	—	—	—	—	+ + +	+ + +	+ + +	—	—	—
M. flavus (196)	—	—	—	—	—	—	—	*M. flavus* (196)	—	—	—	—	—	+ + +	+ + +	+ + +	—	—	—
M. conglomeratus (62)	+ + +	—	—	—	—	—	—	*M. conglomeratus* (62)	—	—	—	—	—	+ + +	+ + +	+ + +	—	—	—
M. roseus (213)	—	—	—	—	—	—	—	*M. roseus* (213)	—	—	—	—	—	+ + +	+ + +	+ + +	—	—	—
M. cinnebareus (349)	—	—	—	—	—	—	—	*M. cinnebareus* (349)	—	—	—	—	—	+ + +	+ + +	+ + +	—	—	—


M. ROSEUS (213)—SERUM
M. roseus (213)
M. aureus (385)
M. albus (238)
M. flavus (196)
M. conglomeratus (62)
M. ureae (88)
M. cinnebareus (349)
M. LUTEUS (426)—SERUM
M. luteus (426)
M. varians (80)
M. aurantiacus (270)
M. albus (238)
M. candidus (401)
M. epidermidis (191)
M. VARIANS (80)—SERUM
M. varians (80)
M. luteus (426)
M. aurantiacus (270)
M. albus (238)
M. candidus (401)
M. epidermidis (191)
M. CASEI (111)—SERUM
M. casei (111)
M. varians (80)
M. luteus (426)
M. aurantiacus (270)
M. albus (238)
M. candidus (401)
M. epidermidis (191)
M. EPIDERMIDIS (191)—SERUM
M. epidermidis (191)
M. varians (80)
M. luteus (426)
M. aurantiacus (270)
M. albus (238)
M. candidus (401)
M. CANDIDUS (401)—SERUM
M. candidus (401)
M. epidermidis (191)
M. albus (238)
M. aurantiacus (270)
M. varians (80)
M. luteus (426)
M. CINNEBAREUS (349)—SERUM
M. cinnebareus (349)
M. roseus (213)
M. aureus (385)
M. albus (238)
M. flavus (196)
M. conglomeratus (62)
M. ureae (88)

closely related in other respects. This is especially noticeable in the light of the results of the general cultural tests of *M. luteus* and *M. varians*. *M. luteus* differs culturally from *M. varians* in nitrate reduction alone, but in spite of this there was a sharp distinction shown by the agglutination reactions.

Of the two types of red coccci for which sera were prepared a considerable amount of group agglutinins appear to be present. *M. roseus* cross-agglutinated with *M. cinnebareus* in a dilution of 1:400, while it agglutinated with its own sera in a dilution of 1:1500. Evidently *M. roseus* and *M. cinnebareus*, altho showing a certain amount of specificity, are closely related serologically.

The results of the agglutination tests as applied to this group appear to be relatively constant, for upon repetition with freshly prepared sera practically the same instances of cross-agglutination together with the large degree of specificity were observed.

AGGLUTINATION ABSORPTION

As has been pointed out by Castellani (1902) and others, organisms may unite with agglutinins but still remain in suspension and fail to show any physical evidence of this union by the flocculation which is characteristic of a positive agglutination reaction. In order to ascertain definitely if the antigen unites with an anti-body in the serum, the agglutination absorption method has been developed and is for very apparent reasons much more reliable and accurate than simple agglutination tests because of the fact that a negative agglutination may give misleading results.

The results of the agglutination absorption tests, as outlined in Table 2, throw very little additional light on the serological relationships of the species of this group. It is evident that only in a few instances does a negative agglutination give misleading results and in such instances only in a matter of a difference of dilution.

Several interesting relationships can be noted from the absorption tests in Table 2. When using *M. aureus* sera, no cross-agglutination could be noted with closely related species in the group, indicating that the orange micrococci represented by *M. aureus* appear to be distinct from the rest of the group. *M. albus* did not appreciably absorb any of the agglutinins from *M. aureus* serum, but *M. ureae* and *M. roseus*, altho failing to give agglutination, showed some absorbing action upon the *M. aureus* serum.

The white strains show practically the same specificity from the standpoint of absorption as were seen in the agglutination. The agglutinins from *M. ureae* serum were absorbed in a greater number of cases by other members of this group than was true of any other of the sera. This is not entirely confined to agglutination absorption as evidences of cross-agglutination between *M. ureae* and other species can be seen in Table 1.

M. candidus and *M. epidermidis*, which are so similar culturally, showed very little tendency to cross-agglutinate, but from the standpoint of absorption test these two species appeared more closely related serologically. *M. epidermidis* when used as an antigen will absorb agglutinin from *M. candidus*, but *M. candidus* when used as an antigen does not appear to absorb agglutinins from *M. epidermidis* serum.

The failure of *M. aureus* to cross-agglutinate with *M. varians* is misleading in light of the agglutination tests, as it was found that a certain amount of cross-absorption was evidenced in nearly all cases.

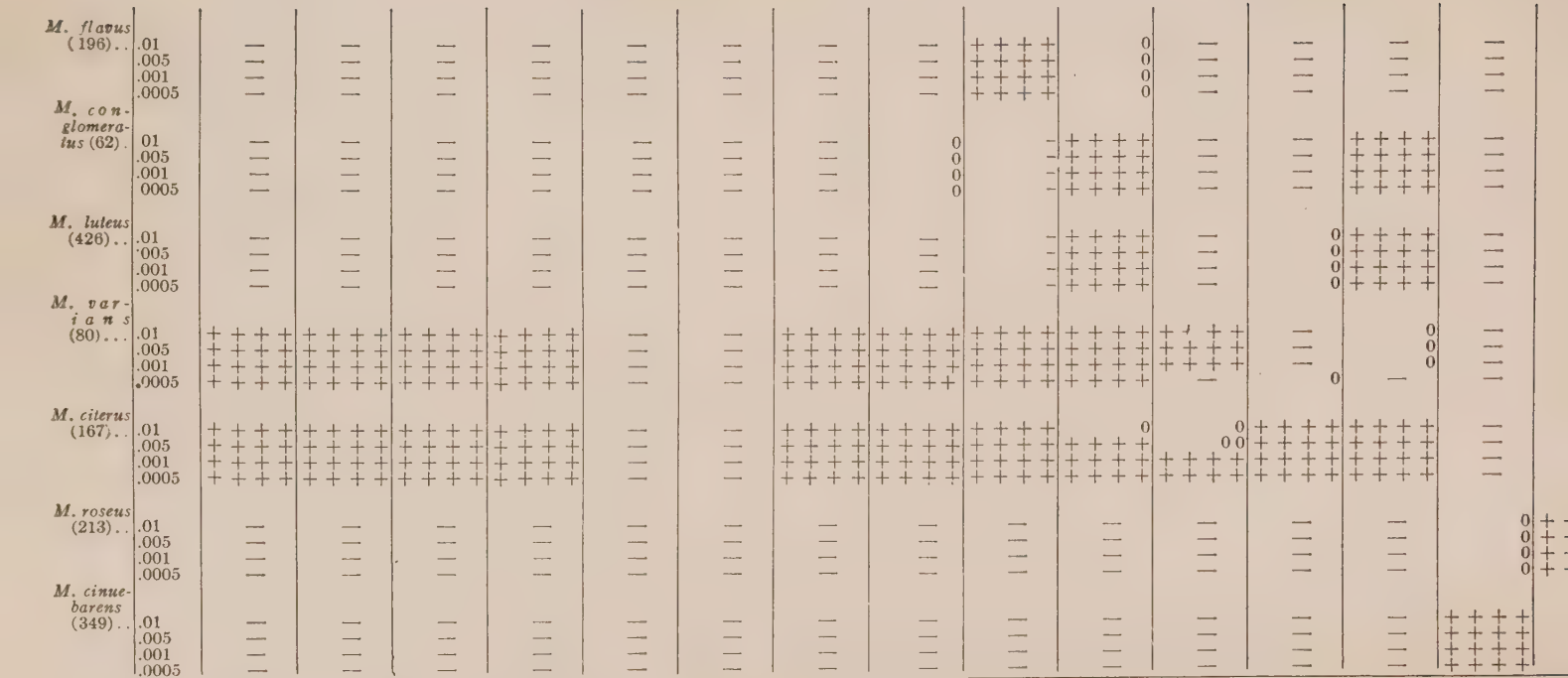
It was also noted in the absorption tests that *M. roseus* and *M. cinnebareus* showed a large amount of cross-agglutination with other species of this group. *M. cinnebareus* antigen absorbed agglutinins from *M. roseus* serum, while *M. roseus* antigen absorbed agglutinins from *M. cinnebareus* serum only in a slight degree.

COMPLEMENT FIXATION

Altho agglutination and agglutination absorption will in most instances give reliable results, serologists are agreed that the fixation or deviation of complement is the most specific and accurate means of serologically differentiating various strains of bacteria. The application of this test to this group has given some very interesting as well as definite results. In the above work dealing with the agglutination absorption considerable cross absorption has been noted. However, in the results of the complement fixation the specificity of the various cultures has been very pronounced. A study of Table 3 will show immediately that very little if any cross fixation was evident even between species which have heretofore been considered very closely related. *M. aureus* and *M. albus* in no instance evidenced any cross fixation, nor did *M. aureus* and *M. aurantiacus* show any tendency to cross fix complement. As has been pointed out in an earlier section of this report (Technical Bulletin No. 102), this particular character

TABLE 3.—CROSS COMPLEMENT FIXATION OF THE SPECIES OF MICROCOCCI.

SERA	AM'T OF SERUM	ANTIGEN														
		385	238	270	191	401	484	88	111	62	196	167	426	80	213	349
M. aureus (385)..	cc.															
	.01	*0	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	0	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
	.005	0	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
	.001	0	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
	.0005	0	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
M. auran-tiacus (270)..	.01	+ + + +	+ + + +	0	+ + + +	+ + + +	—	—	—	+ + + +	—	+ + + +	—	+ + + +	—	—
	.005	+ + + +	+ + + +	0	+ + + +	+ + + +	—	—	—	+ + + +	—	+ + + +	—	+ + + +	—	—
	.001	+ + + +	+ + + +	0	+ + + +	+ + + +	—	—	—	+ + + +	—	+ + + +	—	+ + + +	—	—
	.0005	+ + + +	+ + + +	0	+ + + +	+ + + +	—	—	—	+ + + +	—	+ + + +	—	+ + + +	—	—
M. albus (238)....	.01	+ + + +	0	+ + + +	+ + + +	—	0	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	—	+ + + +	—	—
	.005	+ + + +	+ + + +	+ + + +	+ + + +	—	0	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	—	+ + + +	—	—
	.001	+ + + +	0	+ + + +	+ + + +	—	0	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	—	+ + + +	—	—
	.0005	+ + + +	0	+ + + +	+ + + +	—	0	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	—	+ + + +	—	—
M. epider-midis (191)..	.01	+ + + +	+ + + +	+ + + +	+ + + +	—	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	—	0	—	—
	.005	+ + + +	+ + + +	+ + + +	+ + + +	—	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	—	+ + + +	—	—
	.001	+ + + +	+ + + +	+ + + +	0	—	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	—	+ + + +	—	—
	.0005	+ + + +	+ + + +	+ + + +	0	—	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	—	+ + + +	—	—
M. candidus (401)..	.01	—	—	—	+ + + +	0	—	—	—	—	—	—	—	—	—	—
	.005	—	—	—	+ + + +	0	—	—	—	—	—	—	—	—	—	—
	.001	—	—	—	+ + + +	0	—	—	—	—	—	—	—	—	—	—
	.0005	—	—	—	+ + + +	0	—	—	—	—	—	—	—	—	—	—
M tetra-genus (484)..	.01	—	0	—	—	—	0	—	—	—	—	—	—	—	—	—
	.005	—	0	—	—	—	0	—	—	—	—	—	—	—	—	—
	.001	—	0	—	—	—	0	—	—	—	—	—	—	—	—	—
	.0005	—	0	—	—	—	0	—	—	—	—	—	—	—	—	—
M. ureae (88)...	.01	—	—	—	+ + + +	—	—	0	+ + + +	—	—	—	—	—	—	—
	.005	—	—	—	+ + + +	—	—	0	+ + + +	—	—	—	—	—	—	—
	.001	—	—	—	+ + + +	—	—	0	+ + + +	—	—	—	—	—	—	—
	.0005	—	—	—	+ + + +	—	—	0	+ + + +	—	—	—	—	—	—	—
M. casei (111)..	.01	—	—	—	+ + + +	—	—	+ + + +	0	—	—	—	—	—	—	—
	.005	—	—	—	+ + + +	—	—	+ + + +	0	—	—	—	—	—	—	—
	.001	—	—	—	+ + + +	—	—	+ + + +	0	—	—	—	—	—	—	—
	.0005	—	—	—	+ + + +	—	—	+ + + +	0	—	—	—	—	—	—	—



*0 = No hemolysis, indicating a fixation of complement. + + + + = Complement hemolysis. — = Test not tried.

of specificity from the standpoint of fixation of complement, together with the differences in action upon gelatin, has served as the basis for the separation of *M. aurantiacus* from *M. aureus*.

Of the white forms, one particular point may be noted, namely, the cross fixation in all dilutions between *M. albus* and *M. tetragenus*. This observation is interesting from the fact that *M. tetragenus*, altho producing scanty white growth on agar slants, has very little in common culturally with *M. albus*. Many investigators have felt that it was sufficiently distinct from *M. albus* and related species to place it in an entirely different genus. However, this cross fixation seems to indicate that it is probably a type which should be included in the genus *Micrococcus* as one of the more strictly parasitic species.

M. epidermidis, which as has been pointed out above differs from *M. candidus* only in the matter of nitrate reduction, shows certain results in the complement fixation similar to those noted in the agglutination and absorption tests. In the lower dilutions certain amounts of cross fixation were noted. The relationship between *M. luteus* and *M. varians*, which are comparable species among the yellow forms of this group, did not appear to be so striking.

CONCLUSIONS

It is evident from the foregoing results that the 15 species of micrococci studied in this investigation, with one exception, are serologically distinct. The possible exception is due to the fact that *M. albus* and *M. tetragenus* showed a certain amount of cross-agglutination and cross complement fixation. However, the morphological and cultural differences between these two organisms indicate that they are separate and distinct species.

The specificity of the various sera prepared for the species of micrococci studied would indicate that the chief value of the results of a serological study are from the standpoint of differentiating species and not from the standpoint of separating larger groups.

The large amount of group agglutinins evidenced in many instances would also indicate that this group contains a number of species which are more or less closely related and bears out the conception that all the irregular mass-forming cocci should be placed in one genus.

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